Original Research Article

Hepatitis B Virus Genotypes and Liver Function Tests in Chronic Hepatitis B Patients

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Abstract

Introduction: Hepatitis B virus (HBV) infection is a major public health problem worldwide. Almost 296 million people around the world are suffering from chronic hepatitis B with addition of 1.5 million cases per year. Chronic HBV infection causes morbidity and mortality mostly from chronic liver diseaseslike cirrhosis and Hepatocellular carcinoma (HCC). At least ten HBV genotypes have been identified so far and genotype D and A are commonly found in India. **Materials and Methods:** Two years cross sectional study was done and a total of 78 patients were included in this study. Among all 53 were HBsAg positive. All the processes of diagnosis were carried out at Biochemistry and Microbiology section of Central Laboratory (an ISO 15189:2012 certified and NABH accredited laboratory), Shri Mahant Indiresh Hospital and Central Molecular Research Laboratory (CMRL) [a BSL-III laboratory], Department of Biochemistry, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIMHS), Patel Nagar, Dehradun (U.K). **Results:** Out of 78 subjects, only 53 were positive for HBsAg and of these, DNA was detected only in 43 cases. The genotype D (53.48%) was the most common finding followed by genotype A (23.33%) and genotype F was found in lowest number (2.33%). No significant associations were established between HBV genotypes and Liver function tests (serum ALT, AST, serum Albumin and total serum Bilirubin). **Conclusion:** This study concludes that no significant associations were found between HBV genotypes and liver function tests in chronic hepatitis B patients in north India.

Key Words: HBV, genotype, ALT, AST, LFT.

Introduction

World Health Organization (WHO) estimates that 296 million people were living with chronic hepatitis B infection in 2019, with incidence rate of 1.5 million per year. In 2019, hepatitis B caused an estimated 820 000 deaths, due to the acute or chronic consequences mostly from cirrhosis and hepatocellular carcinoma [1].

Hepatitis-B virus (HBV)infection is a major public health problem worldwide. In 2015, globally, 1.34 million deaths were caused by viral hepatitis, out of which 887,000 deaths were estimated to be due to complications of chronic HBV infection alone. Mostviral hepatitis deaths in 2015 were due to chronic liver disease (720 000deaths due to cirrhosis) and primary liver cancer (470 000 deaths due tohepatocellular carcinoma).Out of the 36.7 million persons living with HIV in 2015 globally, it was estimated that 2.7million had chronic HBV infection.

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In patients of HIV with coinfection with viral hepatitis, liver disease was the major cause of morbidity and mortality [2].A meta-analysis of published data indicates that the prevalence of chronic HB virus infection in India is 1.46% with an estimated 17 million chronic carriers [3].

The frequency of people infected with chronic hepatitis B infection varies worldwide and is highest in the Western Pacific Region and the African Region, where 116 million and 81 million people, respectively. Sixty million people are infected in the Eastern Mediterranean Region, 18 million in the South-East Asia Region, 14 million in the European Region and 5 million in the WHO Region of the Americas [1].

Hepatitis B virus (HBV) is transmitted through contact with blood or body fluids of an infected person and is 50 to 100 times more infectious than HIV. In highly endemic areas, hepatitis B is most commonly spread from mother to child at birth (perinatal transmission) or through horizontal transmission (exposure to infected blood), during the first 5 years of life. The development of chronic infection is common in infants infected from their mothers or before the age of 5 years. It is also spread by needle-stick injury, tattooing, piercing and exposure to infected blood and body fluids, such as saliva and menstrual, vaginal and seminal fluids. Transmission of the virus may also occur through the reuse of contaminated needles and syringes or sharp objects either in health care settings, in the community or among persons who inject drugs. Sexual transmission

is more prevalent in unvaccinated persons with multiple sexual partners. Hepatitis B infection acquired in adulthood leads to chronic hepatitis in less than 5% of cases, whereas infection in infancy and early childhood leads to chronic hepatitis in about 95% of cases. This is the basis for strengthening and prioritizing infant and childhood vaccination [1].

HBV is 42nm DNA virus belonging to the hepadna virus family. The DNA is partially double stranded and contains 3200 nucleotides with overlapping coding regions, leading to several major open reading frames [4, 5]. It is generated through reverse transcription from a longer RNA (approximately 3.5kb, generally referred as pregenomic RNA or pgRNA)[6].

At least of 10 hepatitis B virus (HBV) genotypes (A-J) of hepatitis B virus have been identified so far. All genotypes are with distinct geographic distributions. Several HBV mutants, including pre-core/core promoter mutations and pre-S/S deletion mutations, have been recognized and are predictive of liver disease progression and are also associated with response to antiviral therapy [7].

The most common genotype in India is D followed by A and C. The identification of genotype is important in prognosis and treatment of patients[8]. The prevalence of HBV genotypesvaries geographically. HBV genotypes A through Hhave been found in the United States, with genotypes A, B, and C being most prevalent one[9].

Patients infected with genotypes C and D carry a higher lifetime risk of cirrhosis and HCC development than genotypes A and B. [7, 10] HBV genotypesmay play an important role in the progression ofHBV-related liver disease as well as response to interferon(IFN)therapy[9].

Some HBV genotypes are further classified as sub-genotypes. HBV sequence is characterized by > 8% nucleotide differences for genotype, and 4%-8% nucleotide differences for sub-genotype. Over 30 related sub-genotypes belonging to HBV genotypes have been determined to date, but the mechanisms of different pathogenic characteristics of HBV genotypes are not known for certain [11].

Serum Alanine Aminotransferase (ALT) level is the most commonly used variable for assessment of liver disease [12].

The aim of this study was to find out association between HBV genotypes and Liver Function Test (LFT).

Materials and methods

Before conducting this study, permission by Institutional Ethical committee was granted. **Study**

Population

In this study, a total of 78 patients were included, out of which 53 were found HBsAg positive and rest 25 were negative for screening test and were taken as control.

Inclusion Criteria

Patients of all age group with HBsAg positive and showing Increased/ normal ALT levels in their blood were included in this study.

Exclusion Criteria

Patients undergoing treatment or has recently taken treatment with Anti-viral Therapy or patients with alcoholic hepatitis or patients with HBsAg positive but co-infection with HIV were excluded from this study.

Study Area

All the processes of diagnosiswere carried out at Biochemistry and Microbiology section of Central Laboratory (an ISO 15189:2012 certified and NABH accredited laboratory), Shri Mahant Indiresh Hospital and Central Molecular Research Laboratory (CMRL) [a BSL-III laboratory], Department of Biochemistry, Shri Guru Ram Rai Institute of Medical and Health Sciences (SGRRIMHS), Patel Nagar, Dehradun (U.K).

Study Type and Duration

The study was a cross-sectional study over a period of two years.

Data Collection

Each patient was individually informed about the nature of study and their participation in the current study. Once agreed, an informed written consent was taken from each participant and 5 ml blood sample was drawn from them with the help of BD syringe and immediately transferred in the BD Vacutainer serum separation tube (SST). Sampling was done taking all aseptic precautions and then serum was separated. Tests were performed as per WHO guidelines and manufacturer's instructions. Separated serum was screened for HBV infection in Microbiology section of Central Laboratory of SMIH and then further processed for Liver function tests (Using Vitros 250, Dry Chemistry Slide Method) in Biochemistry section of Central Laboratory, SMIH and molecular characterization to detect HBV DNA and HBV genotypes as demonstrated by Naito et al [13] in CMRL, Biochemistry Department, SGRRIM & HS, Dehradun.

Data Analysis

Data were analysed by using SPSS 23 software.

Results

In the present study, a total of 78 patients were included applying inclusion and exclusion criteria. Out of 78 patients, 53 were found HBsAg positive and rest 25 were negative for screening test and were taken as control. All 78 blood samples were subjected for biochemical estimations like Liver function tests. In 53 samples, which were screened positive for HBV infection, the HBV DNA load was estimated using RT PCR method on COBAS TaqMan99. Out of 53, HBV DNA was detected only in 43 patients, so genotypes were characterized through A-F only in 43 samples.

In this study it was found that out of 43 cases, genotype D was found in 23 (53.48%), followed by genotype A which was present in 10 (23.33%) cases. Genotype B, C and E were found in 05 (11.62%), 02 (4.65%), 02 (4.65%) cases respectively. Least number, 1 (2.33%) of case had genotype F which is a rare genotype in India.

Table – 1 shows that genotype D was found to be the most prevalent one and was found in 23 (53.48%) patients followed by genotypes A which was present in 10 (23.33%). Least number, 1 (2.33%) of case had genotype F.

In our study it was also noted that out of 43 patients 18 (41.86%) had viral load in range between 1.01 $x10^3$ to1.00 $x10^6$, next major group was of 15 (34.88%) patients and their Viral load was between 1.0 $x10^1$ to1.00 $x10^3$ and rest 10 (23.26%) patients had very high viral load (more than 10^6). All groups had Genotype A and D along with other genotypes, but highest viral loads were found only in the patients with genotype A and D.

Table 2 shows mean and SD of ALT levels of 'tests' and 'control'. With the help of t-test, p-value is calculated which shows significant relationship between ALT levels and HBV positive patients.

Table – 3 shows mean and SD of ALT levels and its association with genotypes detected. Using SPSS version 23, Oneway ANOVA was applied to find out the statistical significance. It was observed that association between HBV Genotypes detected, and ALT level wasnot significant.

Table – 4 shows mean and SD of ALT levels and its association with more prevalent HBV genotype A and D. The SPSS version 23 was used, and Oneway ANOVA was applied to find out the statistical significance. It was observed that association between HBV Genotype A and D and raised ALT level were not significant.

Table – 5 shows mean and SD of other liver biochemical parameters like TSB (Total serum Bilirubin), Aspartate transaminase (AST), Alkaline Phosphatase (ALP) andAlbumin (ALB) and its association with HBV genotypes detected. The SPSS version 23 was used, and Oneway ANOVA was applied to find out the statistical significance. It was observed that association between HBV Genotypes and other parameters were not significant.

Table 6 shows mean and SD of other liver biochemical parameters like TSB (Total serum Bilirubin), Aspartate transaminase (AST), Alkaline Phosphatase (ALP) andAlbumin (ALB) and its association with more prevalent HBV genotype A and D. The SPSS version 23

was used, and Oneway ANOVA was applied to find out the statistical significance. It was observed that association between HBV Genotypes and other liver biochemical parameters were not significant.

Table 1: Genotypes Detected (n=43)

Genotype detected	Cases (n)	Cases (%)
Genotype A	10	23.33
Genotype B	05	11.62
Genotype C	02	04.65
Genotype D	23	53.48
Genotype E	02	04.65
Genotype F	01	02.33
Total	**43**	**100.00**

Table 2: Significance of ALT in Tests and Controls group (n=78)

Parameter	Tests	Controls	t-Value	p-Value	Sig.
ALT(Mean ± SD)	270.51± 426.78	30.80± 11.47	1.99254	0.00878	Significant

Table – 3: HBV genotypes detected and its association with ALT (n=43)

ALT	Sum of Squares	Df	Mean Square	F	p-value	Sig.
Between Groups	835467.166	5	167093.433	0.773	0.575	
Within Groups	7992945.439	37	216025.552			Not significant
Total	8828412.605	42				

Table 4: HBV Genotype A and D, and its association with ALT (n=43)

ALT	Sum of Squares	Df	Mean Square	F	p-value	Sig.
Between Groups	220633.740	1	220633.740	0.859	0.361	
Within Groups	7966722.139	31	256991.037			Not Significant
Total	8187355.879	32				

Table 5: Association between Genotypes detected and other liver parameters like TSB, AST, ALP and ALB

PARAMETERS		Sum of Squares	Df	Mean Square	F	p-value	Sig.
TSB	**Between Groups**	41.639	5	8.328	0.336	0.888	NS
	Within Groups	916.749	37	24.777			
	Total	958.388	42				
AST	**Between Groups**	347927.691	5	69585.538	0.621	0.685	NS
	Within Groups	4147486.170	37	112094.221			
	Total	4495413.860	42				
ALP	**Between Groups**	34524.543	5	6904.909	0.708	0.621	NS
	Within Groups	360679.457	37	9748.093			
	Total	395204.000	42				
ALB.	**Between Groups**	2.199	5	0.440	1.485	0.218	NS
	Within Groups	10.957	37	0.296			
	Total	13.157	42				

Table 6: Association between Genotypes A and D and other liver parameters like TSB, AST, ALP and ALB

PARAMETERS		Sum of Squares	Df	Mean Square	F	p-value	Sig
TSB	**Between Groups**	3.364	1	3.364	0.142	0.709	NS
	Within Groups	734.496	31	23.693			
	Total	737.861	32				
AST	**Between Groups**	46299.409	1	46299.409	0.378	0.543	NS
	Within Groups	3794808.470	31	122413.176			
	Total	3841107.879	32				
ALP	**Between Groups**	7570.013	1	7570.013	0.709	0.406	NS
	Within Groups	331152.957	31	10682.353			
	Total	338722.970	32				
ALB.	**Between Groups**	0.268	1	0.268	0.910	0.347	NS
	Within Groups	9.125	31	0.294			
	Total	9.393	32				
	Within Groups	1253621956717641000000.000	31	40439417958633585000.000			
	Total	1539936851888468500000.000	32				

Discussion

Several studies from around the Globe over Hepatitis B infectivity, its Genotyping, effect of drugs and resistance shown by HBV and progression and clinical outcome of Hepatitis B is already done. Different studies have shown variable results depending upon several factors like geographic distribution of HBV genotype, HBeAg seroconversion and ALT levels.

Few studies from different corners of India were also conducted showed certain similarities and some differences.

HBV genotype A and D are the commonest in Indian population [14,15].

Most of the studies from western side of the world and from China and Japan have shown the prevalence of genotypes B and C and their correlation with clinical outcome. However, there are very few studies done in India to show association between HBV genotype A and D and their clinical outcome.

In this study we found that majority (53.48%) of patients were infected with HBV Genotype D followed by genotype A (23.33%). This result is consistent with previous finding from a study done in northern India [16], one study from western India [17] and two studies from Saudi Arabia [18, 19]. A study done earlier on patients from New Delhi, India also shows similar results[20].This result differ from two other studies done earlier in northern India in which HBV genotype A was most prevalent followed by genotype D [14, 15]. The major reason of the different results might be due to the differential demographic distributionof the HBV-genotypes.

In our study we also found HBV genotype B (11.62%), C (4.65%) and E (4.65%), but in low prevalence. This result is consistent with one study done earlier in northern India [15]. A rare genotype F was also found in our study which was present only in one case accounting 2.33% of total genotypes detected and this result is similar to one study done earlier in northern India [16].

In our study we no significant association between HBV genotype and serum ALT, AST, serum Albumin and total serum Bilirubin (TSB) level found. This finding is consistent with a previous study done in New Delhi, India which also showed non-significant association between Genotype A and D and ALT, AST levels, mean age and HBeAg [18]. Our findings are also similar to a study done in Saudi Arabia where they did not find any significant association between HBV genotype and age, gender, liver function tests, or HBV viral load [13]. In contrast to this, a study done in northern India showed that genotype A was most often associated with raised ALT levels as compared to genotype D [14].

A rare genotypes B, C and E were also found in our study. Finding of these genotypes are consistent with one study done in northern India [15] and genotype C was found in one study in northern India [14]. A rare genotype F was also found in our study. This result is in consistence with a study done in north India, where genotype F was found [16].One reason for these findings may be due to Dehradun and adjoining areas like Mussoorie and Hardwar being tourist destinations, people from all over the world come to visit these places. Other possible reason may be the migration of people from other states of India to Dehradun and adjoining areas like Hardwar and Mussoorie in search of job. In an era of frequent international travel and human migration, introduction of new HBV genotype to a community might have far reaching effects, including recombination between genotypes or replacement of one genotype by another. However, there is an urgent need to explore other possible reasons for this unusual prevalence of genotype F. This suggests that genotype F may be indigenous to certain pockets of North India, clustering in and around western UP and Haryana. This unusual finding apparently contradicts the conventional knowledge that HBV genotype closely mirrors ethnic and geographical migration.

Conclusion

From our study we can conclude that, HBV genotype D is the most prevalent one followed by genotype A in India.There is no significant association between various genotypes of HBV and Liver function tests [serum ALT, AST, serum Albumin and total serum Bilirubin (TSB)].Emergence of genotype F in India needs further study regarding its severity, clinical implications and treatment modalities. Since this was time bounded, the sample size was limited. Such type of larger longitudinal studies needed to be undertaken to reinforce the findings.

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